

# 종양특이 cDNA microarray를 이용한 상피성 난소암에서의 유전자 발현 변화

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## Gene expression profile changes in epithelial ovarian carcinomas using tumor-specific cDNA microarray

Hyung Gueon Cho, M.D., Ph.D., Jae Hoon Kim, M.D., Ph.D., Yung Ok Yoo, M.D., Ph.D.,  
Dong Choon Park, M.D., Ph.D., Gu Taek Han, M.D., Ph.D., Jun Mo Lee, M.D., Ph.D.,  
Dae Hoon Kim, M.D., Ph.D.

*Depart of Obstetrics and Gynecology College of Medicine, The Catholic University of Korea, Seoul, Korea*

To identify new bio-markers as well as potential targets for new drugs for epithelial ovarian cancer (EOC), we compared the gene expression profiles of cancer tissues from 25 EOCs with human ovarian surface epithelium (HOSE) using in-house cDNA microarray specified to EOC. Based on a comprehensive method and information from the National Cancer Institute (NCI) Cancer Genome Anatomy Project (CGAP), the cDNA library was constructed. After excluding the overlapping clones, 768 spots were included in the array. We identified the genes and expressed sequence tags (ESTs) (30 up-regulated and 34 down-regulated) that are differentially expressed in EOC tissues. To confirm the expression data, we performed real time RT-PCR experiments. Using microdissected EOC tissues and cell lines, we investigated the expression status of the NET-1 gene, clusterin gene, and actin-binding LIM protein 1. The information provided here will be useful for identifying genes whose products might serve as molecular signatures for the biomarkers of EOCs.

**Key Words:** cDNA microarray, Epithelial Ovarian Cancer, NET-1, Clusterin, Actin-binding LIM protein

### INTRODUCTION

Ovarian cancer is the leading cause of mortality from the gynecologic malignancies (Jermal et al, 2004). Every year, 2,000 ovarian cancer cases are being newly diagnosed and approximately 1,000 deaths, secondary to the malignancy, occur annually in Korea (Ministry health and welfare,

2001). Epithelial ovarian cancer (EOC), the most common form of human ovarian cancer, is usually diagnosed at stage III or IV, a stage at which the 5-year survival rate is below 30% compared with a 95% survival rate for stage I (Jermal et al, 2004). To improve patient survival, the identification and characterization of the key bio-markers that participate in ovarian carcinogenesis would be the essential steps.

Presently, lots of molecular knowledge has been piling up in the oncologic field. Based on the knowledge of differentially expressed genes in

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E-mail: dhkim2k@catholic.ac.kr  
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ovarian cancers, we are beginning to understand the biological behavior of the ovarian cancer cells. Several genes have been considered as candidates for the bio-markers of EOC, such as p53 (Aunoble et al, 2000), K-ras (Alvarez et al, 2001), ErbB2 (Leng et al, 1997), and Myc (Itkes et al, 1990). Moreover, many genes have recently been included as a candidate gene, such as DOC-2 (Mok et al, 1998), and kallikrein 4,5,8,9 (Kim et al, 2001; Yousef et al, 2001; Kishi et al, 2003; Yousef et al, 2003). However, the inconsistency for expression of the genes shows poor or incomplete understanding about EOC tumorigenesis. Moreover, researchers haven't found good parameters for distinguishing the subtypes of EOC and their biological behaviors.

To arrive a better understanding of efficiently the genetic events that contribute to EOC, it has been suggested to identify those genes that are differentially expressed, and that may be directly related to tumorigenesis. cDNA microarray is known to be a powerful tool to identify numerous differentially expressed genes during the neoplastic progression of EOC (Ono et al, 2000; Wong et al, 2001; Jazaeri et al, 2003; Yang et al, 2003). It allows a number of potential candidate genes to be selected for subsequent analysis and applications, such as prostasin (Mok et al, 2001), EP-CAM (Kim et al, 2003), and osteopontin (Kim et al, 2002). However, because this gene expression analysis have been performed on arrays containing a limited set of genes, more studies are needed to characterize EOC.

Recently, by using a small and specialized chip that includes only the genes relevant to the biological process of interest, we can get the advantage of statistical power and eliminating

noise (Sawiris et al, 2002).

In this study, we report on how we employed a home-made cDNA microarray specified to EOC to investigate genes differentially expressed in EOC.

## MATERIAL AND METHODS

### 1. Preparation of the ovarian Tumor-specific cDNA microarray (OVCA Chip V1.0)

To make the ovarian tissue-specific cDNA array, we used a comprehensive human ovarian tumor cDNA library and clones sequenced by the National Cancer Institute (NCI)s Cancer Genome Anatomy Project (CGAP). A comprehensive human ovarian tumor cDNA library in the Lambda ZAP-CMV vector was custom-made using the mRNA samples obtained from 64 ovarian tumor tissues of different histological subtypes, grades, and stages (Stratagene, La Jolla, CA). To prepare the DNA of the lambda cDNA clones, the library was plated onto agar plates consisting of 0.7% top agarose. Agar plugs containing well-isolated single bacteriophage plaques were picked and placed into 1 mL suspension medium (0.1 M NaCl, 8 mM MgSO<sub>4</sub>, 50 mM Tris.Cl, pH 7.5, 0.1% gelatin) with one drop of chloroform. More than 500 single plaques were harvested. After an overnight elution at 4°C, the phages from each single plaque were incubated with E. coli XL1-Blue MRF cells and plated again. Plate lysates of the individual cDNA clones were harvested and the lambda DNA samples were extracted from these clones using the Qiagen Lambda System and the cDNA inserts were PCR-amplified with T7 and T3 primers. After checking their sizes and integrity on 1% agarose gels, the cDNA clones were sequenced by using ABI

PRISM BigDye Termination Cycle Sequencing on an ABI PRISM 310 Genetic Analyzer. Three hundred and fifty unique sequences were so obtained.

The National Cancer Institute (NCI) Cancer Genome Anatomy Project (CGAP) has determined the gene expression profiles of normal, pre-cancerous, and cancer cells by generating more than 400 CGAP libraries and sequencing thousands of clones from each library. The CGAP web site allows researchers to search and analyze all CGAP libraries and other cDNA libraries. Using the cDNA xProfiler software at the CGAP website, we were able to identify 9 ovarian tumor libraries that were derived from microdissected ovarian tumor cells. A total of 7,706 sequenced clones from these 9 different CGAP microdissected ovarian tumor libraries were purchased from Incyte Genomics, Inc (Palo Alto, California) (Table 1).

By comparing the gene expression between these pools of ovarian tumor cDNA libraries with the rest of the cDNA libraries (~6000), a list of 294 genes that are unique to these 9 ovarian tumor libraries was identified. From the sequencing analysis, 12 of these clones have similarity to the Alu repeat and 8 of these clones are no longer in

the unigene database.

Using these 294 ovarian unique expressed sequence tags (ESTs) from the CGAP, 452 known genes and ESTs from our own ovarian tumor library and the normal HOSE cDNA library generated from microdissected normal HOSE cells, 19 house keeping genes, and 3 arabidopsis spike control, we have constructed an ovarian specific cDNA microarray composed of total of 768 spots. The clones were rearranged into two 384 well plates, PCR amplified and purified. The PCR products were re-sequenced and printed onto GAPS slides (Corning Inc., Fountain valley, CA) in duplicate using a PixSys 5,500XL arrayer (Cartesian Technologies, Irvine, CA). A typical result is shown in Fig. 1.

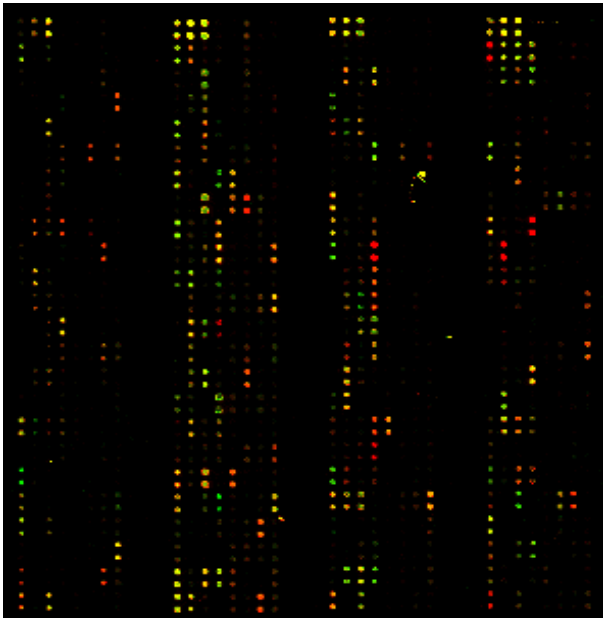
## 2. Gene expression analysis of 25 ovarian tumors

We used this ovarian specific microarray to analyze the expression profiles of 25 ovarian tumors that included 9 serous grade III, 4 serous grade I, 4 mucinous, 4 clear cell and 4 endometrioid histological subtypes. The relevant clinical features of these patients are summarized

**Table 1.** CGAP microdissected ovarian tumor libraries

| Library name  | No.of unique genes <sup>a</sup> | Characteristics (microdissected tumors)                  |
|---------------|---------------------------------|--|
| NCI_CGAP_Ov26 | 98                              | ovary papillary serous carcinoma                         |
| NCI_CGAP_Ov31 | 39                              | ovary invasive tumor                                     |
| NCI_CGAP_Ov32 | 118                             | ovary invasive tumor                                     |
| NCI_CGAP_Ov33 | 17                              | ovary borderline (LMP)                                   |
| NCI_CGAP_Ov34 | 24                              | ovary borderline (LMP)                                   |
| NCI_CGAP_Ov36 | 19                              | ovary borderline (LMP)                                   |
| NCI_CGAP_Ov37 | 1                               | ovary early stage papillary serous carcinoma             |
| NCI_CGAP_Ov39 | 31                              | ovary papillary serous ovarian metastasis to the omentum |
| NCI_CGAP_Ov40 | 74                              | ovary endometrioid ovarian metastasis to the omentum     |

<sup>a</sup> Some of the unique genes are present in more than one of these libraries.



**Fig. 1.** Quantitation of differential hybridization signals between normal ovarian surface epithelial cells (green) and ovarian cancer cells (red) from a home-made ovarian specific cDNA microarray with 768 genes printed in duplicate. Analysis was done with software Imagen 4.2. Yellow means almost equal signals from both samples. One microgram of total RNA from tumor or HOSE cells was labeled with the MICROMAX TSA system.

in Table II. A pool of 6 independent human ovarian surface epithelial primary cell cultures was used as the reference RNA. The TSA indirect labeling method was adopted in this study because of the limited amount of total RNA from the samples. Briefly, 1  $\mu$ g of total RNA from tumor sample was labeled with biotin-dUTP during cDNA synthesis, while the reference RNA was labeled with fluorescein-dUTP. After hybridization, the signals from the tumor and reference sample were amplified with tyramide-Cy3 and tyramide-Cy5 respectively in the presence of horseradish peroxidase. After hybridization, the slides were scanned with a dual laser scanner, ScanArray 3,000 (General Scanning, now part of Perkin Elmer Life Sciences, Boston, MA). The

hybridization signals were analyzed with software Imagen 4.2 (Biodiscovery Inc., Marina Del Rey, CA) and the hybridization signals were normalized to the median signal of 19 house-keeping genes. In addition, another method of data analysis developed by Tseng et al (2001) was also used. In briefly, for comparing two different conditions using cDNA microarray data, a quality index and calibration experiments for normalization were used. A rank invariant method was suggested to select non-differentially expressed genes and to construct normalization curves in comparative experiments. After normalization, the residuals from the calibration data are used to provide prior information on variance components in the analysis of comparative experiments.

Hierarchical clustering was performed using J-Express (Version 2.1).

### 3. Cell lines and culture conditions

All cell lines and cultures were maintained at 37°C in a humidified 5% CO<sub>2</sub> ambient air atmosphere. They were grown in Medium 199 and MCDB 105 (1:1) (Sigma, St Louis, MO) supplemented with 10% fetal bovine serum (Gemini Bio-Products, Calabasas, CA). Normal human ovarian surface epithelial cells (HOSE) cultures were established by scraping the surface of the ovary, as described previously (Tsao et al, 1995). In brief, the scraped cells were spun down, resuspended and cultured in 2.5 mL of growth medium. Cells at 75% confluenc were then harvested by trypsinization and used for the total RNA isolation. For real time PCR, we prepared 9 HOSE cells and 21 ovarian cancer cell lines. Normal HOSE cells used in this experiment were

HOSE601, HOSE667, HOSE696, HOSE 713, HOSE718, HOSE 726, HOSE730, HOSE806, and HOSE831. Ovarian cancer cell lines were used ALST, CAOV3, DOV13, OVCA3, SKOV3, OVCA429, OVCA432, OVCA433, OVCA633, OVCA680, OVCA702, OVCA5071, PEO4, TOV112, TOV21, 2008PO4, MCAS, RMUG-L, RMUG-S, RMG-1 as well as ES-2. MCAS, RMUG-L and RMUG-S were originated from ovarian mucinous cystadenocarcinoma. TOV112 was originated from ovarian endometrioid adenocarcinoma. TOV21, RMG-1 and ES-2 were originated from clear cell carcinoma. The others were from serous cystadenocarcinoma. OVCAR-3, SKOV-3, and ES-2, MCAS, RMUG-L, RMUG-R and RMG-1, PEO4, and 2008PO4 were purchased from American Type Culture Collection (Rockville, MD), the Japanese Collection of Research Bioresources (Tokyo, Japan), Imperial Cancer Research Fund, and the University of Texas Southwestern Medical Center, respectively. The other cell lines were kindly supplied from the Laboratory of Gynecologic Oncology, Brigham and Women's Hospital.

#### 4. Tissue samples

All the patients were treated at Saint Vincent's Hospital between 1995 and 2000. We randomly retrieved the patients' tissue specimens having ovarian tumor with different histologic types and grades based on the WHO and the International Federation of Gynecology and Obstetrics criteria. All tumor tissues were collected from the primary sites of ovarian cancer. They contained less than 20% normal tissue.

For frozen sections, fresh specimens collected at the operating room were placed in tissue culture

medium, Medium 199 and MCDB 105 (1:1) with 10% FBS, and then quickly transported to the laboratory. After removing the nontumorous tissues, the specimens were immediately embedded in Tissue Tek OCT medium (Miles, Elkhart, IN), snap-frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  until use for laser capture microdissection.

#### 5. Laser capture microdissection (LCM)

For real time PCR, microdissected tissues were used. Tissues stored in Tissue Tek OCT medium at  $-80^{\circ}\text{C}$  were sectioned at 7 mm in a cryostat (Leica, Allendale, NJ). The sections were mounted onto uncoated glass slides and immediately fixed in 70% and 50% ethanol for 30 seconds in each concentration, then stained with Hematoxylin and Eosin, and next dehydrated in an increasing series of alcohol and clear xylene for 5 minutes in each concentration. The slides were air-dried once for 3 minutes, and next the sections were laser microdissected with the PixCell II (Arcturus, CA). Morphologically malignant epithelial ovarian cancer cells were so procured.

#### 6. Real-time quantitative RT-PCR

RNA was extracted from the cell lines and tissues using a micro RNA extraction kit as described by the manufacturer (Stratagene, Valenica, CA), and quantified by fluorometry (Gemini Bio-Products). Real-time PCR was performed in duplicate using primer sets specific for NET-1 (forward primer: 5'-CCAGAATGGA CCTGCCCTTT-3'; reverse primer: 5'-TGGTCCCTA TCTAGCCCCAA-3'), ABLIM (forward primer: AACCATAATGCAGAGTGGGCC-3'; reverse primer:

CACAACCCATCCCTCCAGC-3'), clusterin (forward primer: 5'-TTCCCACACTTCTGACTCGGAC-3'; reverse primer: 5'-AACATCCACATCTCACTCC TCC-3') and a house keeping gene, GAPDH (forward primer: 5'-CATCTCTGCCCCCTCTGCTGA-3'; reverse primer 5'-GGATGACCTTGCCCA GCCT-3'), in an ABI PRISM 5,700 Sequence Detector. mRNA was extracted from normal HOSE cell cultures, ovarian carcinoma cell lines and normal ovarian tissues, respectively.

cDNA was generated from 1 g total RNA using the TaqMan reverse transcription reagents containing 1X TaqMan RT buffer, 5.5 mM MgCl<sub>2</sub>,

500 mM dNTP, 2.5 mM random hexamer, 0.4 U/mL Rnase inhibitor, and 1.25 U/mL MultiScribe reverse transcriptase (PE Applied Biosystems, Foster City CA) in a 100 µL. The reaction was incubated at 25°C for 10 minutes, 48°C for 30 minutes and finally at 95°C for 5 minutes. A total of 0.5 µL of cDNA was used in a 20 µL PCR mix containing 1X SYBR PCR buffer, 3 mM MgCl<sub>2</sub>, 0.8 mM dNTP, and 0.025 U/mL AmpliTaq Gold (PE Applied Biosystems, Foster City, CA). Amplification was then performed with denaturation for 10 minutes at 95°C, followed by 40 PCR cycles of denaturation at 95°C for 15

**Table 2.** Clinical characteristics of 25 cases of epithelial ovarian cancers used in the microarray study

| Sample No. | Pathologic Subtype | Grade | Stage | Survival* |
|------------|--------------------|-------|-------|-----------|
| 309        | Endometrioid       | 3     |       | D         |
| 316        | Endometrioid       | 3     |       | D         |
| 321        | Serous             | 3     |       | D         |
| 324        | Serous             | 3     |       | D         |
| 329        | Endometrioid       | 1     |       | D         |
| 332        | Serous             | 3     |       | D         |
| 333        | Serous             | 1     |       | L         |
| 334        | Serous             | 3     |       | D         |
| 341        | Clear              | 3     |       | D         |
| 343        | Mucinous           | 3     |       | D         |
| 349        | Serous             | 3     |       | L         |
| 351        | Serous             | 3     |       | L         |
| 354        | Serous             | 1     |       | L         |
| 373        | Serous             | 1     |       | L         |
| 375        | Serous             | 3     |       | L         |
| 380        | Serous             | 3     |       | D         |
| 382        | Endometrioid       | 2     |       | L         |
| 386        | Serous             | 3     |       | L         |
| 432        | Clear              | 3     |       | D         |
| 436        | Mucinous           | 1     |       | D         |
| 440        | Mucinous           | 2     |       | L         |
| 489        | Serous             | 1     |       | D         |
| 622        | Clear              | 3     |       | D         |
| 700        | Mucinous           | 2     |       | L         |
| 857        | Clear              | 2     |       | D         |

\* D: death, L: live

seconds and annealing/extension at 60°C for 1 minute. The ABI 5,700 system software monitored the changes in fluorescence of SYBR Green I dye in every cycle and the threshold cycle (CT) for each reaction was calculated.

The relative amount of PCR products generated from each primer set was determined based on the threshold cycle (CT) value. GAPDH was used for the normalization of RNA used. Its CT value was then subtracted from that of each target gene to obtain a DCT value. The difference (DDCT) between the DCT values of the samples for each gene target and the DCT value of the calibrator was determined. The relative quantitative value was expressed as  $2^{-DDCT}$ . To confirm the specificity of the PCR reaction, PCR products were electrophoresed on a 1.2% agarose gel.

## 7. Statistical Analysis

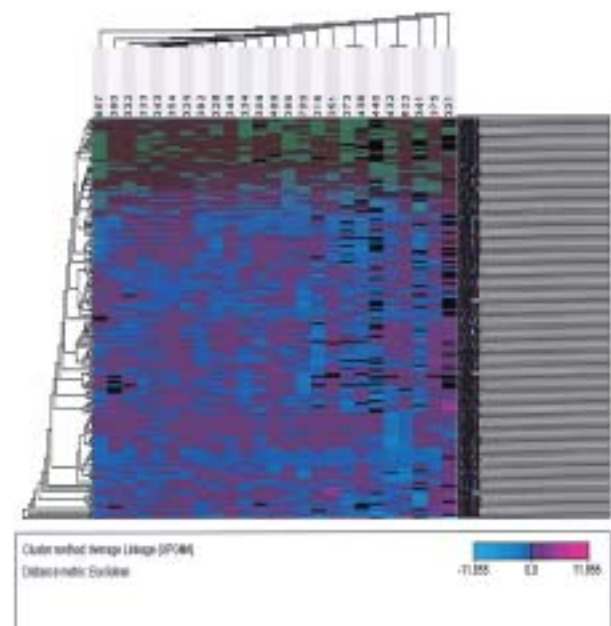
Mann-Whitney U-test was used to test statistical significance for the real time PCR. The level of critical significance was assigned at  $p < 0.05$ . The data were mainly summarized as means and standard deviations (SD). All analyses were performed using SPSS version 9.0 (SPSS Inc., Chicago, IL).

## RESULTS

### 1. Identification of genes differentially expressed between EOC tissues and HOSE cells

The cDNA microarray technique was applied to analyze the expression patterns of EOC tissues and HOSE cells. Among the relevant 763 genes and expressed sequence tags (ESTs) assembled in the

cDNA microarray, 64 were found differentially expressed with the difference signal intensity ratio of more than 2.0 by a Mann-Whitney U test. Genes and ESTs were divided into two groups on the basis of the difference in signal intensity. Out of 64 differentially expressed genes or ESTs, 30 were up-regulated (a signal intensity ratio of more than 2) and 34 down-regulated (signal intensity ratio of less than 1/2) in EOC tissues compared with HOSE cells (Table 3, 4). The data were then visualized using the J-express program, and this process resulted in a dendrogram of genes grouped together on the basis of the similarity of each gene's expression pattern (Fig. 2).



**Fig. 2.** Hierarchical clustering of microarray data. Twenty five ovarian tumors, which include 9 serous grade III, 4 serous grade I, 4 mucinous, 4 clear cell, and 4 endometrioid histological subtypes were used. A pool of 6 independent human ovarian surface epithelial primary cell cultures was used as reference RNA. Red indicated genes that were up-regulated in tumors compared to normal, and green also indicated genes that were down regulated in tumor compared to normal. Data were analyzed using J-Express version 2.1.

## 2. Real-time RT-PCR analysis

To further validate the microarray-based findings, we performed real time RT-PCR experiments with the NET-1 gene, clusterin gene,

and actin-binding LIM protein 1. The results confirmed the pattern of expression identified by the microarray hybridization and showed higher expression of the genes in most of the EOC cell lines and tissues (Fig. 3, 4, 5).

**Table 3.** Genes and ESTs up-regulated in epithelial ovarian tumor<sup>#</sup>

| Gene name                          | Gene No.* | Fold <sup>†</sup> |
|------------------------------------|-----------|-------------------|
| NET-1                              | AF065388  | 49.8              |
| VK1                                | S59162    | 6.2               |
| Ephrin-A1 (EFNA1)                  | M004428   | 4.8               |
| Ribosomal protein L30              | L05095    | 4.3               |
| FLJ 22875fis                       | AK026528  | 4.1               |
| Seb4B                              | X75315    | 3.6               |
| EST                                | Hs.229831 | 3.0               |
| EST                                | Hs.278748 | 2.9               |
| HDCMD 47P                          | AF068287  | 2.8               |
| EST                                | Hs.278749 | 2.6               |
| EST                                | Hs.278876 | 2.5               |
| HumFibm for fibrillarin            | X56597    | 2.5               |
| EST                                | Hs.278747 | 2.5               |
| CD 68                              | M001251   | 2.5               |
| EST                                | Hs.235247 | 2.4               |
| EST                                | Hs.278862 | 2.3               |
| Ribosomal protein S10 (RPS10)      | M001014   | 2.3               |
| Actin binding LIM protein1 (ABLIM) | M006720   | 2.3               |
| Clusterin (SP-40)                  | M001831   | 2.3               |
| EST                                | Hs.235457 | 2.2               |
| EST                                | Hs.254398 | 2.2               |
| EST                                | Hs.276352 | 2.1               |
| Aldehyde reductase (AKR1A1)        | F112482   | 2.1               |
| EST                                | Hs.176874 | 2.1               |
| EST                                | Hs.41132  | 2.1               |
| EST                                | Hs.177415 | 2.1               |
| FLJ 20203                          | M017710   | 2.1               |
| Zinc finger protein (ZNF9)         | M003418   | 2.0               |
| EST                                | Hs.245672 | 2.0               |
| EST                                | H175651   | 2.0               |

<sup>#</sup> More than two fold, P-values <0.05

\* GeneBank accession number

<sup>†</sup> Median value of expression of tumor compared to HOSE



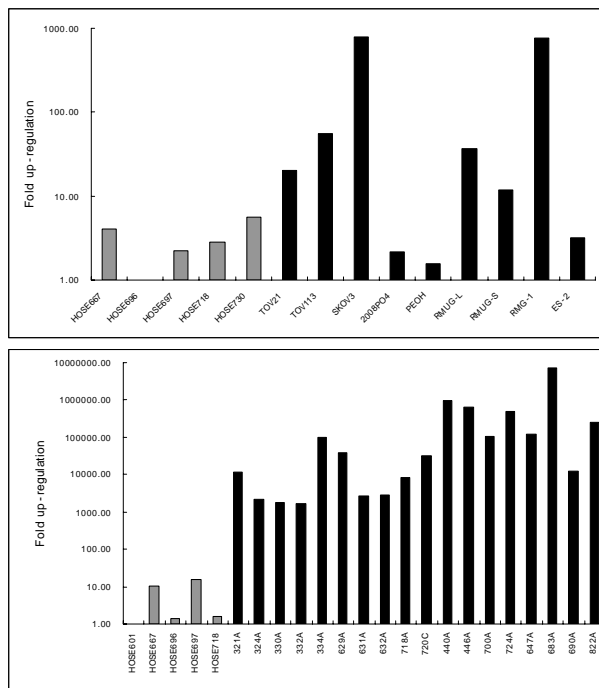
**Table 4.** Genes and ESTs down-regulated in epithelial ovarian tumor<sup>#</sup>

| Gene name  | Gene no.*  | Fold <sup>†</sup> |
|--|------------|-------------------|
| Keratin18 (KRT18)                                  | P000215    | 0.1               |
| LIM protein  | P006448    | 0.1               |
| EST  | P006842    | 0.2               |
| EST  | AX013703   | 0.2               |
| β-galactosidase binding protein                    | P002296    | 0.2               |
| Integrin,β1 (ITGB1)                                | M002211    | 0.2               |
| Iron-responsive regulatory protein                 | M58510     | 0.2               |
| KIAA0109   | D63475.1   | 0.3               |
| Calumenin (CALU)                                   | M001219    | 0.3               |
| Heatshockprotein70 (Hsp70)                         | U69698     | 0.3               |
| Transcriptional repressor (GCF2)                   | gi 3421044 | 0.3               |
| Complement component 3 (C3)                        | gi 4557384 | 0.3               |
| BAC clone RP11-343B21                              | AC009230   | 0.3               |
| Ferritin H chain                                   | gi 182504  | 0.3               |
| EST  | AX018174   | 0.4               |
| Clone DT1P1A7                                      | gi 2781403 | 0.4               |
| Clone 23698  | gi 3360400 | 0.4               |
| Serine/threonine proteinkinase 1                   | gi 5231142 | 0.4               |
| Platelet-type phosphofructokinase                  | D25328     | 0.4               |
| EST  | Hs.274859  | 0.4               |
| Scofilin1 (CFL1)                                   | gi 5031634 | 0.4               |
| I factor (IF)                                      | NM000204   | 0.4               |
| EST  | NP005722   | 0.4               |
| 3BACRP11-297K7                                     | AC009319   | 0.4               |
| EST  | AC034207   | 0.4               |
| Alpha-tubulin isoform1m                            | gi 3420928 | 0.4               |
| RAB7   | M004637    | 0.5               |
| KIAA 0905  | gi 4240298 | 0.5               |
| EST  | Hs.278762  | 0.5               |
| TATA box binding protein (TBP)-associated factor   | M003187    | 0.5               |
| Rab11  | X56740     | 0.5               |
| Eukaryotic translation initiation factor 4 gamma 2 | M001418    | 0.5               |
| EST  | Hs.63472   | 0.5               |
| Actin, gamma1 (ACTG1)                              | M001614    | 0.5               |

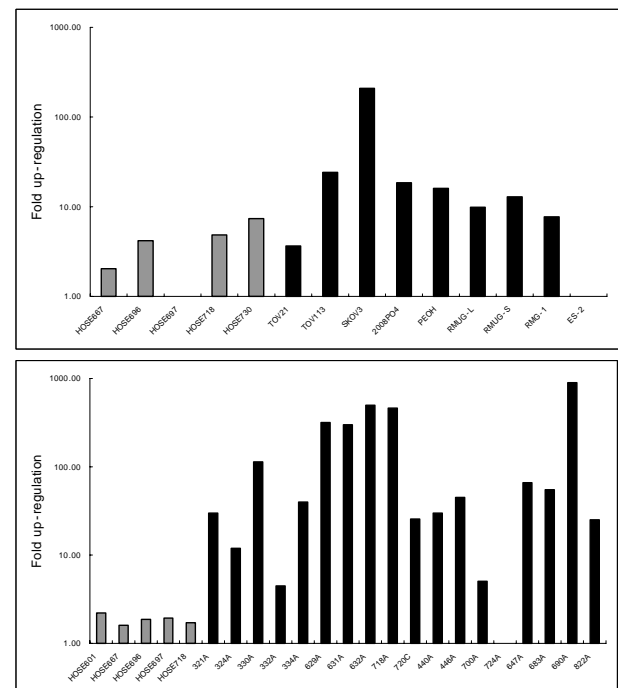
<sup>#</sup> More than two fold, P-values <0.05

\* GeneBank accession number

<sup>†</sup> Median value of expression of cancer to HOSE



**Fig. 3.** Relative quantitation of NET-1 mRNA in HOSE cells and epithelial ovarian cancers. A. Statistically significant differences were not obtained between normal HOSE cells (HOSE 601, HOSE 667, HOSE 696, HOSE 697, HOSE 718) and ovarian cancer cell lines (TOV21, TOV112, SKOV 3, 2008PO4, PEOH, RMUG-L, RMUG-S, RMG-1, ES-2) by the Mann-Whitney U Test. B. Expression differences were statistically obtained between 5 HOSE cells and 18 cancer tissues by the Mann-Whitney U-test ( $p < 0.01$ ). Each value was expressed as the mean of the duplicates.

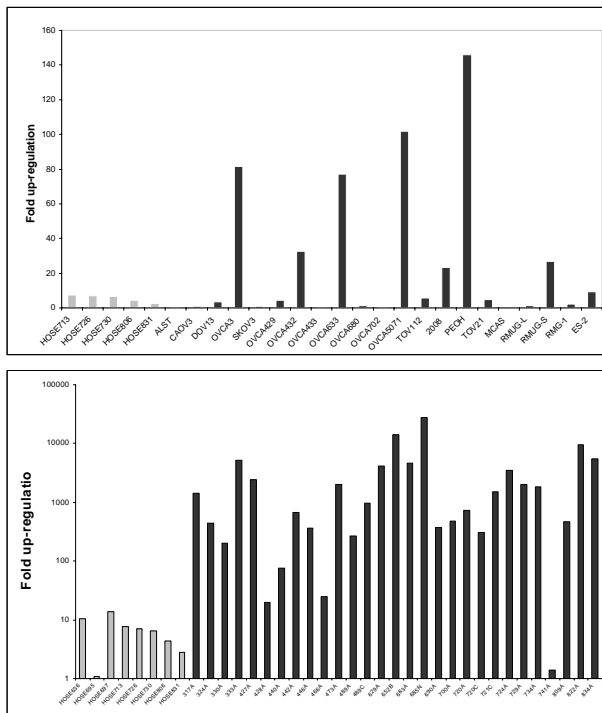


**Fig. 4.** Relative quantitation of ABLIM mRNA in HOSE cells and epithelial ovarian cancers. A. Statistically significant differences were obtained between normal HOSE cells (HOSE 601, HOSE 667, HOSE 696, HOSE 697, HOSE 718) and ovarian cancer cell lines (TOV21, TOV112, SKOV 3, 2008PO4, PEOH, RMUG-L, RMUG-S, RMG-1, ES-2) by the Mann-Whitney U Test ( $p < 0.05$ ). B. Expression differences were statistically obtained between 5 HOSE cells and 18 cancer tissues by the Mann-Whitney U-test ( $p < 0.01$ ). Each value was expressed as the mean of the duplicates.

## DISCUSSIONS

The majority of women with ovarian cancer are diagnosed when they have distant metastatic disease. Primary surgical treatment is seldom curative for late stage disease and adjuvant treatment with radiation or chemotherapy is indispensable. At present, an accurate and reliable, screening method for early detection has not yet been developed. An early detection method that has been suggested is the use of pelvic ultrasound, but this approach has limitations.

Pelvic ultrasound is high in cost and it may lead to unnecessary surgery with its attendant costs and potential morbidity for many non-cancerous cysts of the ovary for every case of ovarian cancer detected (Karlán, 1995). At the same time, there are case reports of ovarian cancers involving only the surface of the ovary that were detected at the time of prophylactic oophorectomy but these lesions had not been seen on the pelvic ultrasounds done shortly before the operation. Although ultrasound undoubtedly remains an important component of the work-up for those



**Fig. 5.** Relative quantitation of clusterin mRNA in normal and malignant epithelial ovarian cancer cell lines and tissues. A. Statistically significant differences were not obtained between normal ovarian epithelial cells (HOSE 713, HOSE 726, HOSE 730, HOSE 806, HOSE 831) and ovarian cancer cell lines (ALST, CAOV3, DOV13, OVCA3, SKOV3, OVCA429, OVCA432, OVCA433, OVCA633, OVCA680, OVCA702, OVCA5071, TOV112, 2008, PEOH, TOV21, MCAS, RMUG-L, RMUG-S: RMG-1, and ES-2) by the Mann-Whitney U Test. OVCA3, OVCA432, OVCA633, OVCA5071, 2008PO4, PEOH, RMUG-S, and ES-2 showed highly expressed clusterin. B. Expression differences were obtained between 8 HOSE cells and 29 cancer tissues by the Mann-Whitney U-test ( $p < 0.01$ ). Each value was expressed as the mean of the duplicates.

women at risk for ovarian cancer, it has not been proved to be an ideal primary screening method. The use of biologic markers is an appealing approach to the early detection because such markers have the potential to detect early ovarian cancers not visible on ultrasound. Blood specimens are easily obtained, acceptable to patients, and could be made inexpensive to assay. There are currently a number of serum markers for ovarian

cancer: CA-125, OVX1, M-CSF, LPA, and folate-binding protein (Bast et al, 1981; Bast et al, 1983, Xu et al, 1993; Suzuki et al, 1995; Suzuki et al, 1998; Xu et al, 1998). While many of these have good prognostic value, none have been proven to be effective in early detection. The best studied of these is CA-125. However, its use in early detection is limited to clinical trials due to its uncertain sensitivity for asymptomatic early stage disease and a lack of specificity in younger women where an elevated CA-125 may be associated with benign conditions including endometriosis, fibroids, pelvic infection, and even menstruation (Woolas et al, 1993). The CA-125 test may be more useful in postmenopausal women. It seems clear that the ideal serologic test for the early detection of ovarian cancer has not yet been developed.

Microarray analysis of ovarian cancer has facilitated the rapid identification of potential diagnostic and therapeutic targets. However, this is just the beginning of a long journey to fully understand the pathogenesis of ovarian cancer. The cDNA microarray analysis of the 768 ovarian tumor-specific cDNA clones with 25 ovarian tumors has demonstrated that tumor-specific genes certainly do exist as potential targets for developing effective bio-markers. A problem with current screening method for early cancer diagnosis is its lack of adequate sensitivity and specificity. If we can validate the tissue specificity of these tumor-specific genes and their roles in the pathogenetic pathways, then we might be able to develop a more specific diagnostic tool for a specific tumor type. Likewise, similar strategies can be applied to other tumors. It will be exciting to identify all the tissue-specific and tumor-specific cDNA clones using the CGAP resources

(Boon et al, 2002), and to use those identified clones for a tumor specific microarray. By coupling with the recent advances in data mining and visualization tools, we might be able to gain a better understanding of the pathogenetic pathways for ovarian cancers. By defining the functional role of each ovarian tumor specific differentially expressed gene into the context of the altered cancer pathways, hopefully, we may one day conquer the scourge of ovarian cancer.

NET-1 is found to be up-regulated by 49.8 fold in EOC tissues compared with the normal control. NET-1 is a new member of the tetraspanin family of proteins. Immunohistochemic analyses of normal cervical epithelium and that of metaplasia, condyloma and CIN of differing severity suggest that NET-1 expression is associated with neoplastic cell proliferation. The potential importance for this gene in cervical carcinogenesis is underlined by an invariably strong expression of NET-1 observed in all the undifferentiated squamous cell cancers examined. (Wollscheid et al, 2002). Our data supports the concept of up-regulation of NET-1 in EOC.

Clusterin is a glycoprotein and it has also been termed apolipoprotein J, and SP40. It is composed of two 35-40 kD subunits ( $\alpha$  and  $\beta$ ) that are encoded for by a single gene (Rosenberg and Silksen, 1995). Clusterin has been proposed to have functions in the setting of tumorigenesis including the regulation of apoptosis (Gleave et al, 2001). However, clusterin has recently been considered as a kind of survival gene because of its overexpression in the surviving neighboring cells compared to dead cells (Sensibar et al, 1995). Moreover, it was shown that clusterin expression

is up-regulated in response to stress agents like heat, cytotoxic drugs, UVA exposure, and oxidative stress (Viard et al, 1999; Dumont et al, 2000). Although the exact role of clusterin is not known, we can recognize the possibility of it being biomarker of EOC.

Actin binding LIM protein 1 is known as a key molecular adaptor with a remarkable potential to mediate protein-protein interactions. Its other ability is related to cofactor exchange that provides a molecular basis for its pleiotropic functions (Matthews et al, 2003).

Many of the candidate genes as molecular bio-markers were screened rapidly in this study. Our in-house cDNA microarray system is an efficient tool to analyze the expression status of the genes in an ovary. The large number of differentially expressed genes identified by this analysis and the characterization of these genes will lead us to develop effective biomarkers as targets for early diagnosis and treatment.

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오늘까지 저의 부족함을 도와 이 논문을 만들 수 있도록 협력해 주신 모든 분들께 영광을 돌리고자 합니다. 특히, 김대훈 지도 교수님, 김수평 전 주임 교수님, 남궁성은 전 주임 교수님, 김진홍 현 주임 교수님, 강남성 모병원 이준모 교수님, 성모병원 한구택 교수님, 영동 세브란스병원 김재훈 교수님, 가톨릭대학교의과대학 병리학교실의 유남진 교수님, 남석우 교수님 그 외 직간접적으로 도움을 아끼지 않으셨던 여러 교수님과 교실 동료, 선 후배님, 연구실 관계자 분께 깊은 감사를 드립니다.

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= 국문초록 =

상피성 난소암의 생화학적 표지물질을 발견하기 위하여, 25개의 상피성 난소암 조직의 유전자 표현 양상을 종양특이 cDNA microarray를 이용하여 비교하였다. Comprehensive method와 National Cancer Institute (NCI)s Cancer Genome Anatomy Project (CGAP)의 유전자 정보를 이용하여, 반복되는 clone을 제외한 768개의 spot으로 구성된 array를 구축하였다. 상피성 난소암 조직에서 상이하게 발현되는 64개의 유전자 또는 ESTs (expressed sequence tags)를 발견하였다. 유전자 발현 여부를 확실하게 알기 위하여 미세조작된 상피성 난소암 조직과 세포주를 이용하여 Real time RT-PCR을 시행하였다. NET-1, clusterin, actin-binding LIM protein 1 유전자의 발현이 상피성 난소암에서 과발현됨을 확인할 수 있었다. 본 연구는 상피성 난소암의 진단에 있어서 molecular target으로 작용될 수 있는 물질을 생성하는 유전자를 밝혀 내는데 유용한 정보를 제공할 수 있을 것이다.

**중심단어:** cDNA microarray, 상피성 난소암, NET-1, Clusterin, Actin-binding LIM protein 1

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